Journal of Forestry Research (2011) 22(1): 59–64
DOI 10.1007/s11676-011-0126-3

ORIGINAL PAPER

Effects of salt-alkali stress on active oxygen metabolism in roots of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame'

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Received: 2009-11-19; Accepted: 2010-03-10

Abstract: Under artificially-simulated complex salt-alkali stress, the levels of active oxygen metabolism in roots were studied using three-year-old cutting seedlings of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame'. The present study aimed at exploring the antioxidant capacity in roots of spiraeas and revealing their adaptability to salt-alkali stress. Results indicate that the oxygen free radicals contents, electrolyte leakage rates and MDA contents in roots of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' show an increasing tendency with the increases of the salinity and pH value, whereas the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) all increased firstly and then decreased. With the increase in intensity of salt-alkali stress, the CAT activity in roots of *Spiraea* × *bumalda* 'Gold Flame' is higher and the increasing extents in the oxygen free radicals contents, electrolyte leakage rates as well as MDA contents are lower compared with *Spiraea* × *bumalda* 'Gold Mound', indicating that *Spiraea* × *bumalda* 'Gold Flame' has a stronger antioxidant capacity.

Keywords: active oxygen metabolism; roots; salt-alkali stress; *Spiraea* × *bumalda* 'Gold Mound'; *Spiraea* × *bumalda* 'Gold Flame'

Introduction

Roots are the main organs for plants to absorb water and nutrient, and they also earliest sense for the soil salt-alkali stress. In the condition of salt-alkali stress, the oxygen supply in roots is insufficient, which induces the increases of reactive oxygen species (ROS) such as $O_2^{\bar{\cdot}}$, and H_2O_2. Excess ROS in cells leads to lipid peroxidation and protein damage, and finally causes plant injury and even death (Blokhina et al. 2000; Narayanan et al. 2005). The plant antioxidant enzyme system consisting of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) plays important roles in eliminating ROS and preventing plants from free radicals injuries caused by salt and other stresses. The increases in their activities are important factors for plants to enhance salt-tolerance ability (Horemans et al. 2000). Studying the levels of active oxygen metabolism in roots under salt-alkali stress has important significance in research of plant salt-tolerance mechanism. However, previous studies on plant salt-tolerance are mainly focused on crops and forages, and the studies on salt-tolerance of flowering shrubs are relatively few.

Spiraea × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' were first introduced and cultivated by Beijing Botanical Garden from American Minnesota State Behre Nursery in 1990. They have been planted and applied more widely (Li 2007) after more than ten years generalization in China. *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' are *Spiraea* foliage shrubs, which have many excellent characteristics, including excellent flowering, strong adaptability, cold tolerance, drought resistance, leanness tolerance, easy propagation, etc. The colored-leaves and the longer ornamental time are their special superiorities. Furthermore, piece planting can form the fine colored ground cover, which is very spectacular. Some researches indicated that *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' had certain salt-tolerance ability (Yu et al. 2005; Yao et al. 2009; Liu et al. 2009). To our knowledge, there is no report on the metabolism of active oxygen in their roots under salt-alkali stress. In the present study, we investigated the changing laws of active oxygen accumulation, membrane lipid peroxidation and antioxidant enzymes activity in roots of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' under simulated complex salt-alkali stress. The study aimed at exploring the antioxidant mechanism in roots of spiraeas under salt-alkali stress and revealing their salt-tolerance ability, which will provide theoretical basis for breeding foliage shrubs with salt resistance.

Foundation project: This work is supported by Innovation Team Project of Northeast Agricultural University of P. R. China (CXZ004-3) and Science Foundation of Heilongjiang Province (C2007-16).

The online version is available at http://www.springerlink.com

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Responsible editor: Hu Yanbo

Materials and methods

Materials cultivation

Three-year-old cutting seedlings of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' were provided by the nursery stock base of Shuangxin Gardening Company, Heilongjiang Province, China. They were transplanted into circular plastic pots (20 cm diameter and 20 cm height) containing washed sand in March, 2009, in the sunlight greenhouse of Northeast Agricultural University. The seedlings were sufficiently watered with Hoagland nutrient solution every week.

Methods

Design of simulated salt and alkaline conditions

Artificial simulation experimental program was designed based on the salt components in salt-alkalinized soils in the west of Heilongjiang Province of China (Shi and Wang 2005). Two neutral salts (NaCl and Na_2SO_4) and two alkaline salts ($NaHCO_3$ and Na_2CO_3) were mixed in various proportions and divided into five treatment groups with gradually increasing alkalinity. All treatment groups had a 1:1 molar ratio of monovalent salts (NaCl+$NaHCO_3$) to divalent salts (Na_2SO_4+Na_2CO_3). Within each group, four concentration treatments were utilized, which are 60, 120, 180 and 240 mmol/L, totally 20 complex salt-alkali treatment groups were simulated with varying salinities and pH values (Table 1). The pH values of 20 treatment solutions and one nutrient solution were measured with a PHS-3BW pH meter. Considering the slight differences in pH values within a group, the mean pH value of four concentrations in a group was taken to represent this treatment group.

Table 1. Salt composition, molar ratio, and pH value of solutions for irrigation in different treatments

Treatment	Salt composition and molar ratio				pH value of various salinity treatments (mmol/L)			
	NaCl	Na_2SO_4	$NaHCO_3$	Na_2CO_3	60	120	180	240
Control	0	0	0	0	6.87	6.87	6.87	6.87
A	1	1	0	0	7.12	7.16	7.20	7.28
B	1	2	1	0	7.93	8.04	8.13	8.24
C	1	9	9	1	8.50	8.66	8.79	8.85
D	1	1	1	1	9.41	9.62	9.73	9.88
E	9	1	1	9	10.21	10.35	10.42	10.45

Stress treatments

In mid-May, when leaves were fully unfolding, the uniformly growing seedlings of two varieties were selected, and respectively randomly divided into 21 groups with three replicates for each group. One group was used as control; the remaining 20 groups were treated with various stress treatments. Control plants were maintained by watering with nutrient solution only; while the stressed plants were watered at 9-10 A.M. with nutrient solution adding various proportions of salts. The treatment solution of 1 L was thoroughly watered to plants in per pot for three times. In order to prevent the salinity outflow, plastic trays were placed under the pots. The exudative solution was returned to the corresponding pots.

Physiological index measurements

At around the hour 9-10 A.M. on the third day after stress treatment, the roots were cut and washed with water, then sipped up with filter paper. The cut roots in each treatment with three replicates were well mixed. Cell membrane permeability, MDA content, oxygen free radicals content and POD activity were determined by using the methods of Gao (2006), SOD activity by the method of Chen and Wang (2002), CAT activity by the method of Bailly et al. (1996).

Statistic analysis

Data was analyzed by Microsoft Excel 2003 and the statistic program SPSS 13.0. Effects of salinity, pH value and their interaction on different strain indices of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' were analyzed by using one-way variance analysis (ANOVA) and Duncan's new multiple range test (MRT).

Results and discussion

Oxygen free radicals content

$O_2^{\cdot-}$ is the main product of oxygen metabolism during the growth process of plants. Therefore ROS content can reflect the change of active oxygen in plants under salt-alkali stress. Fig. 1 shows that, the ROS contents in roots of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' increased with the rising salinity and pH value, and were all higher than that of control. The ROS increment tended to be higher at the higher salinity and pH value. ROS increment in roots of *Spiraea* × *bumalda* 'Gold Mound' at 240 mmol/L salinity and pH value 10.36 was the greatest, which was 36%. Moreover, root ROS contents of *Spiraea* × *bumalda* 'Gold Mound' were significantly higher than those in *Spiraea* × *bumalda* 'Gold Flame' (p<0.01).

Membrane lipid peroxidation

The changing laws of electrolyte leakage rates and MDA contents in roots of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame' were similar under salt-alkali stress (Fig. 2). Electrolyte leakage rates and MDA contents gradually increased with the rising salinity and pH value, and the increments were higher at the higher salinity and pH value. The increments of *Spiraea* × *bumalda* 'Gold Mound' were significantly greater than those in *Spiraea* × *bumalda* 'Gold Flame'. The electrolyte leakage rates of these two species at 60 mmol/L salinity and pH value 9.41 were higher than 50%. The electrolyte leakage rates and MDA contents at 240 mmol/L salinity and pH value 10.36 were the greatest. The electrolyte leakage rates were

87.9% and 80.41%, respectively, in roots of *Spiraea* × *bumalda* ‘Gold Mound’ and *Spiraea* × *bumalda* ‘Gold Flame’. The MDA contents of these two species were increased by 0.426 and 0.38 mmol·g^{-1}FW, respectively, as compared with the control.

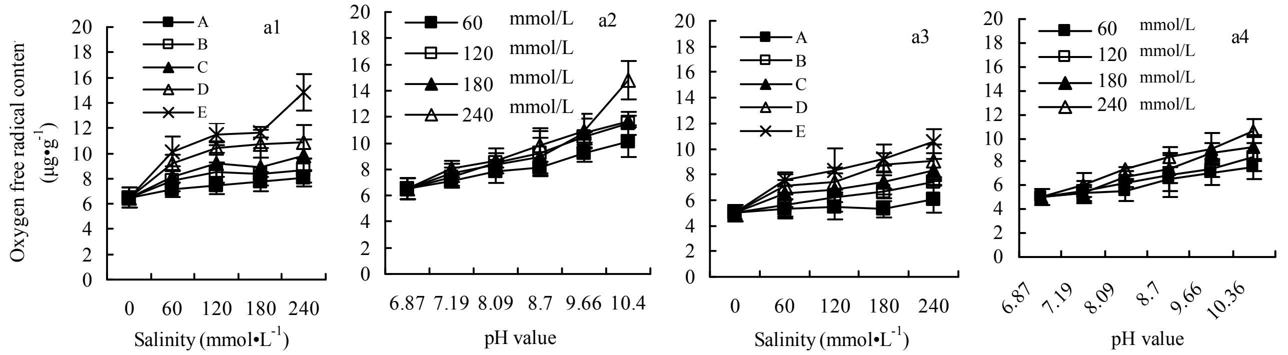

Fig. 1 Effects of salt-alkali stress on oxygen free radicals contents in roots of *Spiraea* × *bumalda* ‘Gold Mound’ (a1, a2) and *Spiraea* × *bumalda* ‘Gold Flame’ (a3, a4). A: pH value 7.12-7.28; B: pH value 7.93-8.24; C: pH value 8.50-8.85; D: pH value 9.41-9.88; E: pH value 10.21-10.45.

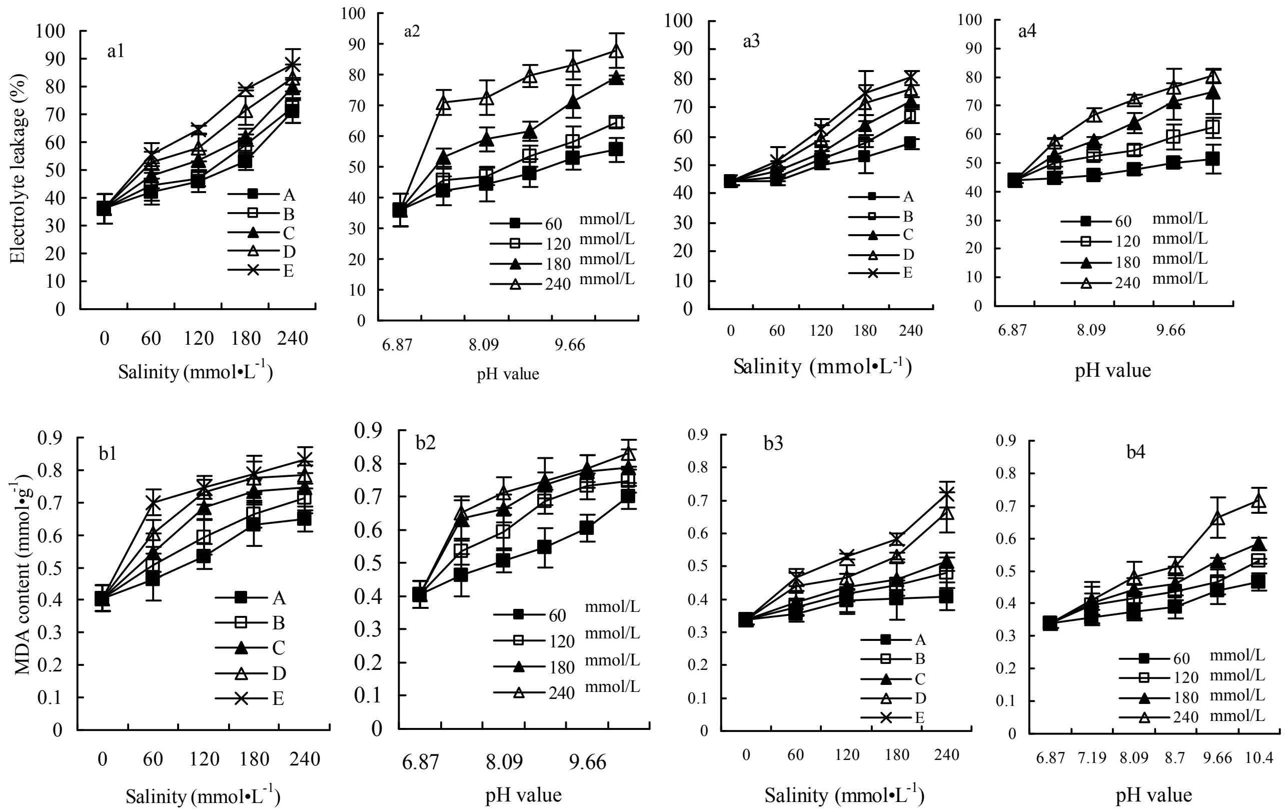

Fig. 2 Effects of salt-alkali stress on the electrolyte leakage rates and MDA contents in roots of *Spiraea* × *bumalda* ‘Gold Mound’ (a1, a2, b1, b2) and *Spiraea* × *bumalda* ‘Gold Flame’ (a3, a4, b3, b4). A: pH value 7.12-7.28; B: pH value 7.93-8.24; C: pH value 8.50-8.85; D: pH value 9.41-9.88; E: pH value 10.21-10.45.

Antioxidant enzymes activity

Fig. 3 shows that, under the salt-alkali stress of lower salinity (< 120 mmol/L) or lower pH value (treatments A and B), the activities of SOD, POD and CAT in roots of *Spiraea* × *bumalda* ‘Gold Mound’ and *Spiraea* × *bumalda* ‘Gold Flame’ basically increased with the rising salinity and pH value. However, under the stress of higher than 120 mmol/L salinity, treatments C, D and E,

the activities of antioxidant enzymes initially increased and then decreased with increasing pH value and salinity. When the activities of antioxidant enzymes began to decrease, the corresponding salinity and pH value were different for *Spiraea* × *bumalda* ‘Gold Mound’ and *Spiraea* × *bumalda* ‘Gold Flame’. The salinity and pH value in *Spiraea* × *bumalda* ‘Gold Mound’ were lower than those of *Spiraea* × *bumalda* ‘Gold Flame’. The differences in activities of SOD and POD were not significant between these two species, but the CAT activity in roots of *Spiraea* × *bumalda* ‘Gold Flame’ was significantly higher than that in *Spiraea* × *bumalda* ‘Gold Mound’ (p<0.01).

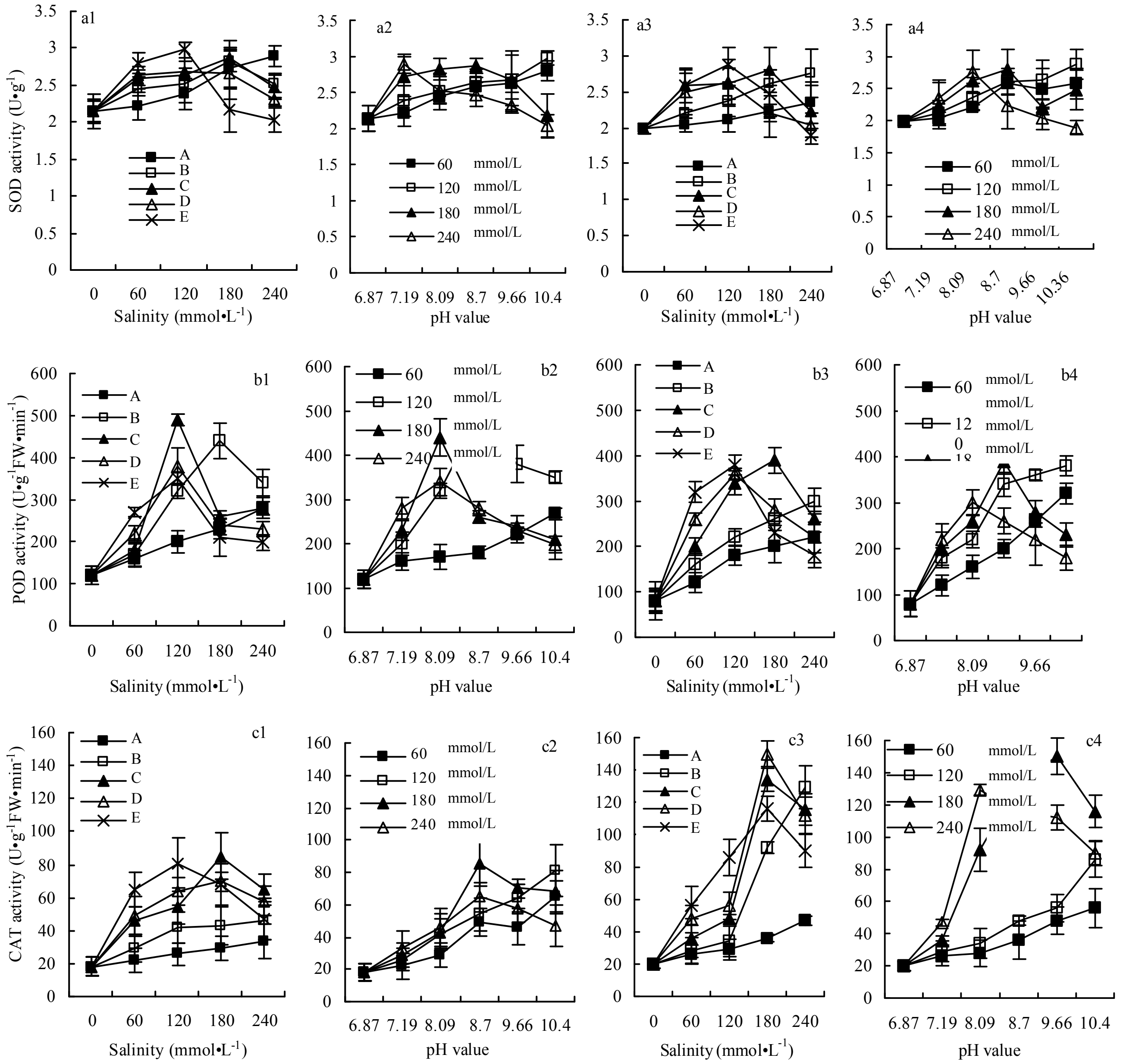

Fig. 3 Effects of salt-alkali stress on the activities of SOD, POD and CAT in roots of *Spiraea* × *bumalda* ‘Gold Mound’ (a1, a2, b1, b2, c1, c2) and *Spiraea* × *bumalda* ‘Gold Flame’ (a3, a4, b3, b4, c3, c4). A: pH value 7.12-7.28; B: pH value 7.93-8.24; C: pH value 8.50-8.85; D: pH value 9.41-9.88; E: pH value 10.21-10.45.

Relationship analysis between strain indices and salinity and pH value in roots of spiraeas

One-way ANOVA analysis showed that the effects of salinity on CAT activity in roots of *Spiraea* × *bumalda* ‘Gold Mound’ and on SOD activity in roots of these two species were not significant (p>0.05) (Table 1). However, the effects of salinity on root ROS content of these two species were significant at 0.05 level, and on the other indices at 0.01 level. The effects of pH value on

all indices in roots of these two spiraeas were significant at 0.01 level except for SOD activity, which was insignificant (p>0.05), indicating that the six strain indices in roots of spiraeas were not only closely related with salinity, but also with pH value. The interaction of salinity and pH value only significantly affected POD activity of these two species and CAT activity in *Spiraea* × *bumalda* 'Gold Flame' (p<0.01), the interaction effects on the other indices were insignificant (p>0.05). It also indicated that the interaction of salinity and pH value had a certain influence on the physiological indices in roots of spiraeas.

Table 2 Salinity, pH value and their interaction influences on different strain indices of *Spiraea* × *bumalda* 'Gold Mound' and *Spiraea* × *bumalda* 'Gold Flame'(*F*)

Strain indices	*Spiraea* × *bumalda* 'Gold Mound'			*Spiraea* × *bumalda* 'Gold Flame'		
	Salinity	pH value	Salinity × pH value	Salinity	pH value	Salinity × pH value
Oxygen free radicals content	3.34*	13.05**	0.55^{NS}	3.42*	7.31**	0.17^{NS}
Electrolyte leakage rate	52.89**	13.82**	0.30^{NS}	36.22**	12.23**	0.79^{NS}
MDA content	14.11**	10.92**	0.21^{NS}	13.46**	14.62**	1.00^{NS}
SOD activity	1.07^{NS}	0.25^{NS}	2.07^{NS}	1.16^{NS}	1.42^{NS}	1.31^{NS}
POD activity	29.39**	9.98**	9.86**	9.64**	12.41**	5.91**
CAT activity	2.35^{NS}	10.38**	0.92^{NS}	76.15**	28.67**	6.98**

*p<0.05; **p<0.01; ^{NS}p>0.05.

Discussion

Under salt-alkali stress, the balance between the production and elimination of active oxygen ions is broken; this imbalance leads to oxidative injuries in plants. The accumulation of active oxygen ($O_2^{\bar{\cdot}}$, H_2O_2, OH·, etc.) causes peroxidation of membrane lipids, an increase in membrane permeability, the exudation of electrolyte and small molecular organic matters. The imbalance of material exchange in cells furthermore results in a series of physiological and biochemical metabolism disturbances, and visual damage of plants at varying degrees (Zhao et al. 2004; Liao et al. 2009). Antioxidant enzyme system (SOD, POD, CAT, etc.) in cells is the main factor preventing plants from the oxidative damage under stress, and its enhanced activity is a normal physiological response for cells to resist stress (Chen et al. 2007). Under the salt-alkali stress, the changes of antioxidant enzyme depend on the varying species as well as the pattern, intensity, and duration of stresses. The defense capability of the entire antioxidase system depends on the coordination of different enzymes (Zhang et al. 2008). MDA, a product of membrane lipid peroxidation, together with electrolyte leakage rate are typically used as indices of membrane damage.

This research indicated that, the active oxygen contents in roots of spiraeas increased with the rising salinity and pH value, the electrolyte leakage rates and MDA contents gradually increased as well. At lower salinity (< 120 mmol/L) or lower pH value (treatments A and B, pH value 7.12-8.24), the activities of SOD, POD and CAT in roots of spiraeas were enhanced. These enzymes timely eliminated the produced oxygen free radicals and reduced the degree of cell membrane injury. The increasing extents of electrolyte leakage rates and MDA contents were low. When the salt-alkali stress was too intense to adapt, the roots of spiraeas were severely injured, the excess accumulation of oxygen free radicals caused the decreases in the activities of antioxidant enzymes. Thus, the degree of membrane lipid peroxidation was aggravated, the integrity of cell membrane in roots of spiraeas was severely damaged, and the electrolyte was massively excluded. Compared with *Spiraea* × *bumalda* 'Gold Mound', the CAT activity was higher and the comprehensive capacity of antioxidant enzyme system was stronger in roots of *Spiraea* × *bumalda* 'Gold Flame', hence, the cell injury degree was reduced. Moreover, the increasing extents of electrolyte leakage rates and MDA contents were less than those in *Spiraea* × *bumalda* 'Gold Mound'. Therefore, the antioxidant capacity of *Spiraea* × *bumalda* 'Gold Flame' was relatively better.

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